Genomic glucocorticoid signaling in the hippocampus: understanding receptor specificity and context dependency

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CHAPTER 6

Summary and general discussion

More than 45 years of research on the effects of glucocorticoids on brain function has yielded many insights (as outlined in the introduction), but also left a number of long-standing questions. One conundrum has been how activation of the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) can lead to very different, or even opposite effects. It also remained unclear how the consequence of activation of a single receptor, GR, can differ from cell to cell and from situation to situation. A mechanistic basis for appropriate changes in gene expression that underlie the adaptive effects of stress steroids is the diversity of MR/GR signaling partners, involving coregulatory proteins and other, non-receptor transcription factors (TFs). In this thesis we have investigated two specific aspects of transcriptional regulation in response to glucocorticoids in the brain: the cause of MR/GR specificity, and the role of crosstalk with other TFs. This final chapter will summarize novel insights from the in this thesis described studies, followed by general discussion of the data, functional and clinical significance and future perspectives.

Summary

The first research chapters (**Chapter 2, 3 and 4**) examined the genomic interactions of MR compared to GR, and the common and specific transcriptional responses mediated by the two receptor types. In **Chapter 2** we used chromatin-immunoprecipitation (ChIP) followed by sequencing (ChIP-seq) to obtain hippocampal genome-wide DNA binding profiles for MR and GR. This was done in brain tissue of adrenalectomized rats that had received an intraperitoneal injection of corticosterone 60 minutes prior to sacrifice. Comparison of MR and GR cistromes resulted in 918 MR-exclusive sites, 1450 GR-exclusive sites and another 475 MR-GR overlapping sites. Of note, the MR binding sites were detected for two different dosages of corticosterone (0.3 mg/kg and 3.0 mg/kg) and, in contrast to our expectations, limited overlap was found between MR cistromes upon the lower and higher hormone concentration. We validated several MR-exclusive target loci by ChIP-qPCR in an independent set of adrenally intact animals, around the time of their endogenous corticosterone peak. Since DNA binding by MR/GR needs consecutive modulation of gene activity to eventually have functional consequences for a (brain) cell, we studied associated transcriptional effects in **Chapter 3**. In order to filter out false positive putative targets, we focused on binding sites that were located within gene bodies or the (proximal) promoter region. Subsets of MR-specific, GR-specific and MR-GR overlapping targets were assessed in a forebrain MR knockout model (fbMRKO). In these mice, a decreased expression was found for a number of predicted MR-specific targets, for the classical glucocorticoid target gene *Fkbp5* and a couple of other overlapping targets, and – surprisingly – for two predicted GR-specific target genes. The most robust effect was observed on mRNA levels of the MR-specific target *Jdp2*. This was (besides the panel of classical targets) the sole MR/GR target that was responsive (i.e. upregulated) in subsequent validation using the model of restraint stress. We thus identified *Jdp2* as a bona fide hippocampal MR-specific target gene.

In the studies described in **Chapter 2** we also examined sequences of the DNA fragments defined by the MR and GR peaks. Virtually all sites bound by MR and/or GR contributed to *de novo* detection of the glucocorticoid response element (GRE). In addition, we were surprised to find that all MR-exclusive sites were associated with an Atoh1 consensus site (part of the group of 'E-box sequences'), which was not retrieved from the GR-exclusive or MR-GR overlapping dataset. Based on their hippocampal expression, we hypothesized NeuroD family members to bind this additional sequence. Using ChIP-qPCR, we could indeed confirm *in vivo* Neurod2 occupancy near MR-exclusive loci. Next, we studied the NeuroD proteins that are expressed in adulthood (Neurod1, Neurod2 and Neurod6)

in reporter assays driven by a promoter that contained a GRE with an adjacent Atoh1 site (GRE-At). These experiments were performed in HEK293 cells, to which expression plasmids for the receptors had to be added as well. All three NeuroD family members were able to potentiate corticosterone-induced transactivation at this construct, for both MR- and, unexpectedly, GR-transfected cells. This effect was not dependent on either the N-terminal or C-terminal part of MR/GR, as demonstrated by the use of truncated versions of the receptors. We explained the *in vitro* lack of specificity for potentiation of MR over GR signaling to be likely a result of the absence of a neuronal-specific chromatin/cellular context, and formed the novel hypothesis that additional factors mediate an indirect effect of NeuroD on glucocorticoid signaling. In **Chapter 4** we aimed to further explore the mechanism behind the NeuroD-mediated enhancement of MR signaling. We first demonstrated by ChIP-qPCR in fbMRKO animals that Neurod2 binding was independent of MR binding. Also GR binding was unaffected by the absence of MR for the target loci tested, except for a slight increase of GR occupancy at the *Per1* promoter. The purpose of following experiments was to find out which part of the NeuroD protein is responsible for its potentiation of glucocorticoid signaling. Various NeuroD-related E-box binders (MyoD, Myf5 and a MyoD truncation) were studied in our (adapted) GRE-At reporter assay. MyoD was able to potentiate MR/GR transactivation when its DNA binding domain was replaced with that of Neurod2, or when the E-box sequence in the luciferase promoter was adjusted to be effectively bound by MyoD. This latter construct was further studied in combination with the several E-box binders. We showed that MyoD variants harboring their domain responsible for chromatin remodeling activity, but lacking an activation function for direct recruitment of the transcriptional machinery, could still enhance MR/GR-mediated transcription. Our overall conclusion was that NeuroD acts permissively to enable MR binding rather than prevent GR binding, and chromatin remodeling seems the main mechanism driving NeuroD potentiation of MR signaling.

The interaction between GR and other TFs has mainly been studied in cell line models. In **Chapter 5** we examined GR context-dependency at a genome-wide scale *in vivo*, in a memory-relevant behavioral model. To this end, we made use of an object location memory (OLM) task in which glucocorticoids can act as a switch for long-term memory formation, but this is dependent on training-induced noradrenergic signaling. One of the TFs activated (i.e. phosphorylated) by noradrenaline is cAMP response element-binding protein (CREB). We therefore assessed the potential interaction of GR with pCREB. In our setup, vehicle-injected animals did not discriminate between objects. Corticosterone-injected animals (3.0 mg/kg, subcutaneous) on the other hand, showed

evident preference for the object in a new location relative to that in the familiar location, serving as a measure of memory. Four treatment groups were examined for DNA binding of the two factors: [1] non-trained vehicle-injected control animals, [2] non-trained corticosterone-injected animals to observe the effect of GR activation, [3] OLM-trained vehicle-injected animals to observe arousal-induced changes in pCREB, and [4] OLM-trained corticosterone-injected animals to observe the effect of combined CREB and GR activation. In each of these groups genome-wide binding of pCREB and GR within the hippocampus, at a timepoint of 45 minutes after the injection, was measured by ChIP-seq. We included the most robust peaks (i.e. those present in 3/4 or 4/4 of the biological replicates) in our analysis. Interestingly, the GRE content of the GR peaks detected in OLM-trained animals was lower compared to the non-trained groups, suggesting that the mode of GR signaling is affected by the training status. Peaks were analyzed for changes between treatment groups. As few as 6 loci were found differentially occupied by pCREB and we decided to focus on the GR binding data in the analysis. Amongst the GR peaks, we found 67 differentially occupied loci, mainly in response to corticosterone treatment. Of these, 20 loci were affected independent of training status, while 27 loci were specific to non-trained animals and 19 loci specific to OLM-trained animals. We subsequently confirmed corticosterone-mediated gene expression changes on pre-mRNA level for the classical target gene *Fkbp5*, as well as newly identified GR targets *Gjb6* and *Nsmf*. Overall, we provided evidence that the GR cistrome, whether or not as a result of interactions with pCREB, can be affected by exposure to a training task.

Towards an updated corticosterone receptor model

1. MR-mediated effects in the higher corticosterone range

Back in 1985 it was shown by Reul and de Kloet that MRs and GRs are differentially distributed in the brain, but colocalized in hippocampal neurons. They also demonstrated that corticosterone has a tenfold higher affinity for the MR than for the GR (1). Since then, we have had the view that MR is occupied by hormone under basal conditions and GR gets bound in conditions with elevated hormone levels. The general assumption has therefore also been that corticosterone concentrations that exceed 'basal hormone levels' lead to GR-mediated effects. In other words: the GR is the receptor for stress-induced increases in corticosterone (2). Later work showed that non-genomic effects mediated by hippocampal MRs require higher corticosterone concentrations (3), but the notion of the 'saturated MR' has held for its genomic effects.

In **Chapter 2** of this thesis we however showed that two high doses of corticosterone which ought to be both super-saturating for MR, could still lead to differences in target gene binding. We observed other binding sites in response to 'very high' 3.0 mg/kg corticosterone compared to 'high' 0.3 mg/kg corticosterone (at which receptors should already be saturated). Unexpectedly, an increased hormone concentration was thus able to induce binding of MRs to additional sites. Apparently not only non-genomic but also genomic MR is sensitive for hormone changes in the stress-range. For novel target *Jdp2* its promoter binding by MR was demonstrated in **Chapter 3** to be accompanied by stress-responsive regulation of the gene.

A possible explanation for the discrepancy between hormone concentrations needed for maximal occupancy of MR and those leading to maximal DNA binding effects may be the transient corticosterone peak applied in our ChIP-seq experiment (4), while K_d determinations take place under steady-state conditions (5). Also the lag between hormone binding and nuclear translocation should be taken into account, as well as the subsequent step of stable DNA binding (6, 7). Finally, there may be signal integration with the lower affinity membrane receptors, which could lead to e.g. changes in MR phosphorylation that might be needed for binding to specific DNA loci (8). In any case, the MR cistrome was clearly affected by higher than 'basal' hormone concentrations, and we should adjust our MR/GR model accordingly. RNA-seq experiments using different corticosterone doses would have to reveal subsequent implications for corresponding target genes.

2. Reaching MR versus GR specificity

Upon the discovery of MR and GR presence in the hippocampus, initial functional findings pointed to complementary effects of MR and GR on behavior (9) and even opposite effects on neuronal excitability (10). However, around the same time the molecular structure of the two receptors was found to be very similar, in particular in the DNA binding domain (11). In accordance, MR and GR can both bind the GRE sequence and concomitantly transactivate genes. The receptors do strongly differ in their capacity to interact with other TFs, e.g. in case of transrepression of AP-1 and NF-κB (12). Also, GR is uniquely capable of repressing transcription via negative GREs (13). However, in ChIP-seq data available from the hippocampus the predominant binding mode of both MR and GR is to GREs. This suggests that the opposite effects mediated via MR and GR on e.g. hippocampal CA1 cells must be caused by receptor-specific GRE-driven target genes.

In **Chapter 2** we indeed describe unique as well as shared loci for MR and GR in hippocampal chromatin. The unique sites all contained GREs in association with other motifs, which presumably bind other TFs that transfer the specificity. This was in fact predicted by K. Yamamoto who found that evolutionary conservation of the GRE predicted functionality (i.e. binding) of that sequence (14). He noted that a receptor molecule does not 'know' whether a sequence is conserved in the DNA of another species. Therefore, the conservation of the 15-nucleotide long GRE likely reflects a larger stretch of DNA which includes binding sites for other TFs, and these confer the actual capacity for receptor binding, and perhaps specificity. This model is supported in a previous study on the hippocampus in which binding sites for SP-1 family members distinguished functional from non-functional GREs with respect to GR binding (15).

The additional motifs associated with GR-exclusive binding in our study were left unexplored, though some potential cross-talk partners were detected in a distinct subset analysis (16). However, for the Atoh/NeuroD motif that we found in all of the MR-exclusive binding sites, we showed actual binding of Neurod2 to these loci. This finding puts MR in a longer list of nuclear receptors that – in a tissue-specific manner – rely on additional TF presence for their binding and/or functionality. For example, mouse liver GRs depend on bHLH protein E47 at many loci (17) and estrogen receptors interact with pioneering factor FoxA1 (18). The NeuroD-MR link is in all likelihood specific for brain MR, but in other MR-expressing tissues similar proteins may provide context specificity at the chromatin level. Our work described in **Chapter 2** suggests that there are additional proteins involved in the specific interaction between MR and NeuroD factors, as in reporter assays in non-neuronal cells also GR activity was enhanced by NeuroD proteins.

Experimental follow-up on the NeuroD-MR link puts challenges. First, there are several NeuroD family members, which all might interact with MR. Second, it is difficult to recapitulate the cellular context of end-differentiated cells, even in iPSC-derived cultures. Knockout of NeuroD factors will interfere with neuronal differentiation (19-21), and this may also happen when NeuroD is inactivated in end-differentiated neurons (given their continued presence). We therefore, in the work described in **Chapters 2 and 4**, used more simple systems with controlled expression of the various factors, and made use of closely related MyoD proteins that bind related E-box sequences. These experiments brought us insights on potential mechanisms of interaction. We could differentiate between domains necessary for chromatin remodeling and direct transactivation, and we have shown that the interaction likely involves addition proteins. In this respect it would be interesting to compare MR and GR complexes with RIME methodology (22), in which

a comprehensive characterization of interacting proteins is achieved. Another attractive option is to study MR and GR interactions with other TFs using proximity ligation assays (23). These approaches should serve to confirm receptor-specific protein interactions, and determine the extent of brain region, cell-type, context, and species specificity of the findings reported in this thesis.

Intriguingly NeuroD is involved in the differentiation of particular neuronal phenotypes, which apparently includes MR function. Even though MR may respond to corticosterone levels in the stress range, as shown in this thesis, its affinity for MR is tenfold higher than for GR. As a result, it sets the sensitivity of the hippocampal circuitry for activation, with consequences for both cognition and mood. Genetic as well as human pharmacological data suggest that a gain-of-function variant of the MR confers resilience to depression, in particular in premenopausal women (24, 25). Current data on potential functional interactions between MR and NeuroD factors are scarce. Elevated Neurod2 levels were found in the ventromedial prefrontal cortex of depressed *men* (26) – it is for now unclear how these factors relate, considering that postmortem studies often have many experimental issues, including the use of medication. Even though, the finding would be consistent with one study suggesting that in males the more active variant of MR increases the risk for depression (27). Neurod2 has been found to be significantly co-expressed with the 5-HT1A receptor in the human brain (28), which is one of the signaling pathways that was controlled by MR in the original studies on rodent hippocampus (29). However, no genetic associations between NeuroD proteins and mood disorders have been discovered to date.

Next to establishing a possible mechanistic basis for MR-specific effects, our data also provide leads to the actual genes and proteins that underlie such effects. Considerable efforts have gone into candidate gene approaches to understand, for example, genes and proteins that drive modulation of CA1 pyramidal neuron excitability (30). Our bottom-up approach of identifying MR-specific loci, and linking these to gene expression resulted in a number of – likely – bona fide MR-specific target genes in the mouse brain. We established for a small number of genes that they were clearly expressed at lower levels in the brains of fbMRKO mice. Combining our ChIP-seq with RNA-seq will in future likely reveal more MR-specific target genes. *Jdp2* mRNA also responded to stress-induced corticosterone elevations, which may be an example of MR-mediated functional effects at concentrations traditionally considered as 'super-saturating'. While we did not formally prove that these genes are not regulated via GR, it will be interesting to evaluate their expression in particular settings. One such setting is exposure to high levels of synthetic

GR-selective glucocorticoids. The suppressed cortisol that accompanies this kind of treatment is predicted to deprive the MR of its ligand. This may actually contribute to the psychiatric side effects of treatment with GR-selective drugs (31, 32), and lower expression of MR target genes would substantiate this notion. The MR-specific target genes that we found may be used in further studies in the context of mood regulation.

3. Binding at MR-GR joint sites

While MR-specific target genes may explain some of the intrinsic genomic MR-mediated effects that are unique to this receptor, it has long been clear that MR and GR have a very similar DNA binding domain, and that they can bind to identical GRE sequences. In fact, canonical GR target genes such as *Gilz* and *Sgk1* were independently characterized as functionally important MR target genes (33, 34). We confirmed that MR binds to the *Fkbp5* gene and observed that *Fkbp5* expression was reduced in the hippocampus of fbMRKO mice. This finding seems quite relevant to those studying the effects of chronic stress on the brain. *Fkbp5* expression is routinely used as a readout for GR activation (35). The protein Fkbp5 is part of the complex that regulates ligand binding and nuclear translocation of GR, and its upregulation by GR provides intracellular negative feedback. It has been proposed to be a mediator of long-term stress effects in the brain (36), in part via methylation of its promoter (37, 38), and Fkbp5 inhibitors are considered for clinical development in psychiatry (39, 40). Our data call for a reevaluation of MR in these effects, including the notion that Fkbp5 may also act as a co-chaperone for factors other than GR.

The regulation of genes via both MR and GR would expand the effective concentration range of corticosterone for these genes by an order of magnitude. From the overlapping binding sites in our ChIP-seq dataset we are not able to tell if these are derived from a combination of MR and GR homodimers binding the same locus in different neurons, or that MR-GR heterodimers (41) were present in our samples. However, using re-ChIP in the hippocampus at particular loci the *in vivo* formation of heterodimers has been made plausible (42). Heterodimerization may also explain why others found that a subset (15%) of hippocampal GR DNA binding sites was also associated with NeuroD factors (43). These likely represent MR-GR overlapping target loci as described in **Chapter 2**, at two of which in **Chapter 4** we have detected Neurod2 binding as well. Besides binding of each heterodimer partner to a half-site of the GRE, co-occupancy of GREs by MR and GR could also be realized via higher order complexes (44), or with MR tethering to GR (6). The DNA occupancy studies do not allow to unequivocally determine whether the outcome of such MR-GR interactions is additive, synergistic, or rather antagonistic. Although hippocampal

GR is upregulated in fbMRKO mice, this apparently could not compensate for the lack of MR transcriptional activity at the several overlapping genes that were downregulated in these animals. It will be interesting to see in functional studies whether the receptors cooperate, counteract or simply have independent effects.

GR interactions during memory consolidation

Since the 1980s we are well aware that GR via corticosterone influences the process of memory consolidation (9, 45, 46), which was later shown to be mediated by transcriptional responses of the receptor (47). In fact, in the setup that we used in **Chapter 5**, corticosterone can act as a switch for long-term memory consolidation. Because administration of beta-blockers prevents the effect of corticosterone (48), we hypothesized that there is a molecular interaction between two downstream effectors of noradrenaline and corticosterone, pCREB and GR respectively. We found limited evidence for such an interaction on the DNA level. For GR binding we did observe a mild context-dependency, while for pCREB differences between groups were almost absent. Future gene expression studies – at multiple timepoints after corticosterone treatment – should determine whether the transcriptional outcome of GR activation is also context dependent.

We worked under the assumption that pCREB and GR would act as a genomic 'coincidence detector' within hippocampal neurons. However, since we assessed whole hippocampi, we cannot exclude dilution effects. *Arc* reporter mice show a clear mosaic activation of neurons after learning experiences, and only in those cells CREB seemed activated (49). Therefore, single cell approaches (50) may yield outcomes that are more in line with our original hypothesis, and show more context dependent changes with respect to pCREB, as well as GR binding. Of course, our hypothesis may also be wrong. The potentiation of learning could alternatively involve noradrenaline-induced GR modification. Other studies showed reduced coimmunoprecipitation of CREB with a GR phosphorylation site mutant (51) and a unique gene regulatory profile of specific GR phospho-isoforms (52). Moreover, because the brain is a network there is the possibility that noradrenaline and glucocorticoids independently affect different neuronal populations, e.g. in amygdala and hippocampus (48). We must conclude that despite the elegance of our behavioral setup with corticosterone as memory switch, our study did not resolve the question of how GR acts differently at the genome in order to facilitate memory consolidation.

Nevertheless, the different experimental conditions tested in the ChIP-seq study of **Chapter 5** might provide us with greater understanding of several psychopathologies. The strengthening of memory consolidation by stress is considered part of the pathogenesis of post-traumatic stress disorder by many (53, 54). Transcriptional changes that depend on co-activation of CREB and GR are of particular interest to this situation. On the other hand, high levels of glucocorticoids per se (i.e. stress without a particular learning context) may be relevant to any stress-related psychopathology, although typically chronic rather than transient exposure is looked at.

Technical considerations and future approaches

The work in this thesis generated new insights, but of course there is much more to do. It is a truism that the design of the experiment determines the outcome. In this light, there seems value in reiterating some aspects of the here described studies. Our DNA binding data were obtained within an hour of corticosterone treatment, but in two very different conditions. In **Chapter 5** we saw that a relatively mild contextual change of training versus control may already affect GR binding. In **Chapter 2** we studied the cistromes of MR and GR in adrenalectomized rats which were at rest. Another recent study did not find any differences in GR binding upon restraint stress compared to similar corticosterone exposure in a non-stressed control situation (43), and the type of stressor as well as the lack of adrenals in those animals might have had a role in that negative finding. Therefore, it will be crucial to tailor future work to specific physiological or pathological contexts. It also needs to be kept in mind that DNA binding does not equal transcriptional activity (55) and in many cases MR/GR occupancy might hold a permissive effect on gene expression rather than having a strong regulatory role on its own. Furthermore, to predict MR-regulated target genes, we have limited ourselves to loci within or very close to genes. Techniques that map the three-dimensional conformation of the genome, such as 4C and Hi-C (56), will support a more careful annotation of binding events and can reveal long-range interactions of loci that affect sites of transcriptional activity further than their nearest gene (4). Even if we were able to identify additional unknown target genes for the MR based on ChIP-seq data, it will be good to combine future ChIP-seq studies with measurements on genomic spatial organization as well as transcriptomics to directly link DNA occupancy to functional binding events.

DNA binding that is associated with transcriptional changes, still brings the issue of timing. For example, in **Chapter 5** we evaluated pCREB binding 45 minutes after training,

and we may have missed transient effects given that changes in transmitter activity occur almost instantaneously (57). On the other hand, we evaluated gene expression by looking at unprocessed transcripts (pre-mRNA) at the same time point, and this in all likelihood is too early to detect many changes. Also time of the day is a relevant factor, as the expression of or occupancy by signaling partners may show circadian variation (58). Ideally, time courses would be constructed both for GR DNA binding and transcriptional responses, but given the budgets necessary for omics studies, the considerations remain difficult when addressing the effects of transiently changing hormone levels.

Since the start of the work described in this thesis there has been an impressive development and implementation of new techniques to assess transcriptional effects and chromatin regulation. Some of these can also be applied *in vivo*. ATAC-seq is one such an approach (59), which may be used to gauge the overall accessibility of chromatin, as a consequence of MR or GR activation. Given the presence of particularly GR in many different neuronal and non-neuronal cells in the hippocampus, it is advisable to reduce cellular complexity before applying such technology. At the proteomics level, RIME is a promising technique (22) to assess proteins that are in the same complex as the receptors, and this may be used to confirm and expand data on other TFs that interact with MR and GR to establish their cell- and context specific effects. Furthermore ChIP-exo has an increased resolution compared to traditional ChIP assays, as the binding site is narrowed down to physically protected bases. However, this comes with the disadvantage of more challenging data analysis because of e.g. increased amount of multiple reads to be mapped to the same locus (60).

Once MR/GR loci and predicted target genes are identified, a next challenge is to determine the contribution of individual genes and proteins to hippocampal functioning. This challenge amounts to creating shortlists from longlists. Combining primary targets (ChIP-seq) with transcriptome data provides a filter, but additional strategies seem necessary to pinpoint targets that can be functionally studied using knockout and knockdown models. Of course, for lack of true shortlists, a biologically informed hypothesis and the availability of mouse models can lead to meaningful results. In this respect it would be interesting to for example test whether mice that lack *Nsmf* (61) display potentiation of memory formation after GR activation.

While the work described in this thesis addressed basic questions, there are immediate applications for clinical research. The established MR target genes may not only be helpful in the context of dexamethasone-induced psychiatric side effects, but also in relation to

chronic exposure to endogenous glucocorticoids during chronic stress and in Cushing's disease. They may also be useful to evaluate the effects of hyperaldosteronism as occurs in Conn's syndrome. Those patients also report psychological disturbances, and these may well involve MR target genes in the brain, either in the aldosterone-selective brain stem neurons or via classical cortisol-preferring MRs as present in the hippocampus. Given that MR gain-of-function seems to protect against affective disorders, the MR-dependent cistrome (and transcriptome) should hold cues to factors that confer resilience to stress-related disorders.

Concluding remarks

We have shown that the dogma of MR saturation and its function being restricted to basal hormone levels is incorrect, since increasing corticosterone does yield additional genomic MR binding. We identified NeuroD as the factor driving MR over GR binding specificity in the hippocampus. Finally, we have explored context dependency of GR genomic action in a model that uses corticosterone as a switch for memory consolidation. More experiments are needed in which hormone effects are determined in relevant experimental settings, such as behavioral tasks related to learning and memory. Combining these with ever expanding databases on the genome, and tissue-specific expression of signaling partners, should speed up our understanding of the role of MR- and GR-dependent signaling in relevant adaptive and pathophysiological settings over the coming years.

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